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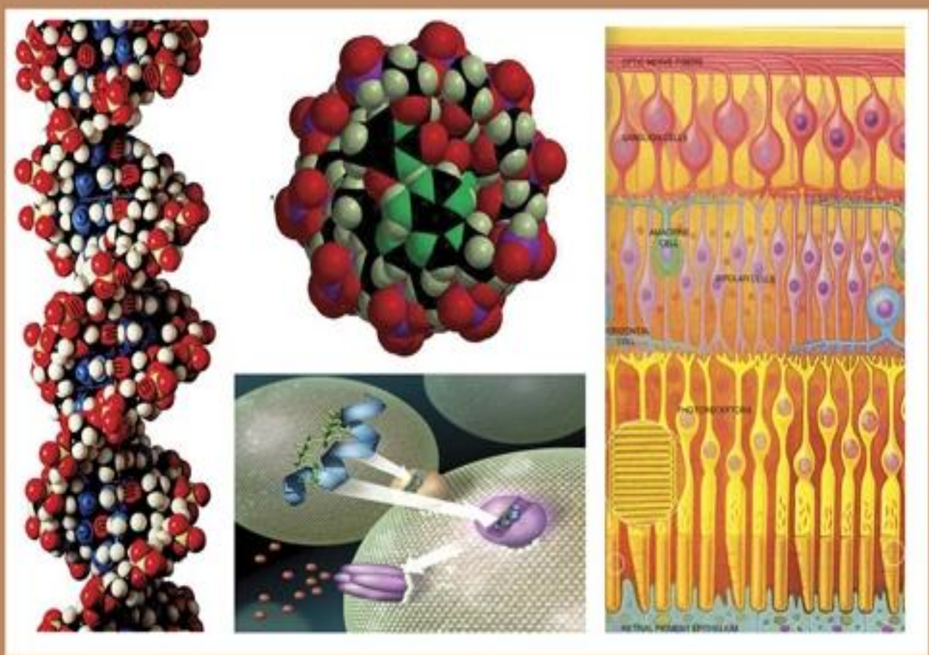

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The Early Predictive Role of Serum Zonulin Level for COVID-19 Severity in Patients Survival

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) is a new infectious disease caused by the SARS-CoV-2 coronavirus, which first appeared in Wuhan and quickly spread around the world. The Middle-East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) outbreaks in 2003 and more recently have demonstrated how lethal CoVs can be when they infect humans across the species barrier. The novel severe acute respiratory syndrome coronavirus 2 has threatened the world in many ways (SARS-CoV-2). Zonulin is a member of a protein family whose first member, pre-haptoglobin 2 (HP2), was discovered nearly ten years ago. (Rittirsch D 2013).**Materials and Methods**: A total of 120 Covid-19 patients' serum samples were collected and an apparently healthy group (n=60) with an age range (of 35-75) years, was admitted from AL-Amal Hospital. Zonulin levels were measured by enzyme-linked immunosorbent assay (ELISA) (kit. Metabolic parameters were measured by enzymatic spectrophotometer methods. The correlation coefficients between serum Zonulin levels and age, BMI, Elements and electrolytes were also evaluated.**Results**: Serum Zonulin, CRP, D-dimer and ferritin levels were significantly higher in Patients with COVID-19 (324.4±12.46) vs in control (79.69±11.77), (42.67±1.84) vs in control (3.36±0.25), (4188.21±198.73) vs in control (289.43±251) and (738±20.09) vs in control (130.66±9.2) (P <0.001). The correlation of Zonulin levels in COVID-19 patients was significantly positive with age, CRP, D-dimer and ferritin levels but negative with Iron, Ca and Na levels. The serum of Zonulin levels in moderate COVID-19 patients significantly high compared with the critical and severe patients group.**Conclusions:** Serum Zonulin levels increased in COVID-19 patients, especially in severe cases. Therefore, Zonulin levels demonstrate a prognostic value for predicting the severity of COVID-19. Continuous Zonulin results throughout the study period revealed that the severe group's values were higher than those of the non-severe group.

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INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was the first new coronavirus to be discovered, and it was discovered in Wuhan, China in December 2019 (Huang C 2020). Because SARSCoV-2 is so contagious, even asymptomatic individuals could spread the virus (Wang D 2020). On February 11, 2020, the World Health Organization (WHO) declared the illness brought on by SARS-CoV-2 to be coronavirus disease 2019 (COVID-19). A number of clinical symptoms, including pharyngalgia, fever, cough, tiredness, anorexia, headache, diarrhea, nausea, vomiting, dyspnea, and even acute respiratory distress syndrome, are present in COVID-19 patients (ARDS). Numerous critically ill or severe patients needed to be admitted to the intensive care unit (ICU).(Fan H 2020) The total death rate for COVID-19 individuals ranges from 2 to 5%, and it may even be greater among the elderly, according to the described clinical characteristics of these patients (Fan H 2020, Chen N 2020). In the early stages of the epidemic, mortality in Wuhan City peaked at about 7% (Lu J 2020). Coronaviruses (CoVs) are enclosed viruses having a positive sense, single-stranded RNA genome. They are members of the family Coronaviridae and the subfamily Coronavirinae (order Nidovirales). (Fauquet CM 2005) CoVs have the biggest RNA viral genomes, with lengths ranging from 26 to 32 kilobases (kb). CoVs have been divided into three classes based on genetic and antigenic criteria: -CoVs, -CoVs, and -CoVs. (Fauquet CM 2005, Pradesh U 2014). Coronaviruses primarily infect birds and mammals, causing a variety of lethal diseases that particularly affect the farming industry (Lee C 2015, Bande F 2015) the regulation of solute and fluid exchange, as well as the absorption of nutrients, the intestinal epithelium's primary functions. (Odenwald 2013). The gut epithelium and its associated components, together with gut-associated lymphoid tissue and the neuroendocrine network, may also act as regulators in the passage of environmental antigens from the intestinal lumen into the submucosa, according to an increasing number of studies (Fasano A 2011). The hypothesized paradigm states that the mucosal barrier is dysregulated, which increases the number of antigens and other macromolecules that enter the host and trigger local and/or systemic inflammation and immune activation. (Vancamelbeke M 2017) The World Health Organization has categorized COVID-19 as a pandemic and attributes it to infection with SARS-CoV-2. SARS-CoV-2 is able to interact with the stomach, despite the fact that it has mostly been demonstrated to affect the respiratory system. Intestinal homeostasis requires the gut barrier to be maintained, and disease can occur when it is compromised. This process attributed to “gut leakiness” influences tolerance and immunity (Sharma L 2020). Using indicators of disease severity and clinical progression in this situation would make it easier to identify individuals who need aggressive management and surveillance early on and would help ensure that healthcare resources are used wisely.

The protein zonulin has been linked to the regulation of mucosal permeability because it is capable of reversible tight junction disassembly (Barbara G 2021). Zonulin has initially identified as an endogenous human counterpart of the intestinal bacterium Vibrio cholera's zonula occludens toxin (Zot), a bacterial enterotoxin (Ahmadi AR 2020). It has been postulated that zonulin activates EGFR through proteinase-activated receptor 2 (PAR2) and G protein-coupled receptor PAR2, which transactivates EGFR, to begin tight junction disintegration. (Ajamian M 2019) These two receptors are activated, which results in a reduction in transepithelial electrical resistance and suggests enhanced intestinal permeability (Barbara G 2021). The finding that Zot activates intracellular pathways that result in actin polymerization driven by protein kinase C shows that cytoskeleton modification contributes to enlarged intestinal permeability (Cenac N 2004). Zot and zonulin are analogues, and that zonulin has a similar activation mechanism. The features of 120 COVID-19 patients admitted to Hubei NO.3 People's Hospital of Jianghan University, Wuhan, thoroughly evaluated in this study.

MATERIALS AND METHODS

The case-control study comprised 120 patients (min.-max.ages:35-75 years) infected individuals with COVID-19 who were admitted to AL- Amal Hospital in the province of AL-Najaf in Iraq. All participants provided informed consent before the study's start between January 2022 to September 2022, after approval by the ethics committee of the Iraqi Ministry of Health and the Environment. These patients confirmed the diagnoses by a chest X-ray, CT scan, and quantitative RT-PCR performed 7 to 12 days following the onset of symptoms to make the diagnosis. The patients were split into three smaller groups: (50) mild/moderate cases of COVID-19 if they had a fever, respiratory symptoms and radiological pneumonia evidence, (41) severe cases and the (29) critical patients after then dead. Patients with COVID-19 were collected at admission and the disease severity was determined using Murray scores (Slifer ZM 2021). If a patient satisfied any of the following criteria, they were judged to have severe COVID-19.

1. (≥ 30 /min) repertory diversion.
2. A saturation level of 90% or less for resting oxygen
3. Arterial oxygen (PaO2) / percentages of inspired oxygen less than 300 mmHg.
4. A repertory failure that necessitates mechanical breathing and an intensive care unit (ICU).

Critical patients who pass away are also regarded as having not survived.

The patients' names, ages, sexes, weights, and heights were entered into a file along with their registration information. Ninety supposedly healthy persons were chosen as the control group. They were similar to the patients in terms of age and sex distribution.

Those who meet the exclusion criteria are smokers, pregnant women, those with systemic immunological illnesses, and volunteers who have thyroid gland disease or any other chronic conditions, such as diabetes, or cardiovascular disease, or who are taking long-term oral corticosteroids. Medical syringes that used to collect five milliliters of venous blood from each patient and control group. Two milliliters were placed in EDTA tubes for complete blood count analysis, and the remaining blood was placed in gel tubes and left at room temperature for 15 minutes for coagulate samples before being centrifuged for 10 minutes at (3000 Xg) to provide the serum. The sera were separated and stored in Eppendorf tubes at (-20 C0) until a biochemical experiment could be performed. Using an auto hematological analyzer, full blood count values were evaluated (linear, Spain). By using an enzyme-linked immunosorbent test (ELISA) (Melsin, Chain), the levels of Zonulin assays were found in serum samples. This was often done within the first 24-48 hours after admission. Furthermore, high-density lipoprotein cholesterol (HDL-Cholesterol), triglycerides, and total cholesterol (TC) (HDL-C). Na, K, and Ca electrolytes concentrations were determined using kits for colorimetric techniques (Agappe). Zn and Fe were measured using kits for colorimetric techniques (Agappe). Fluorescence immunoassay, or FIA, was used to measure the levels of serum ferritin and D-dimer (ichromaTM).

Statistical Analysis:

The Statistical Package of Social Science (SPSS) version 26 and (Statistical Package for Social Sciences) package program.. The mean and standard deviation were used to express continuous variables (SD). For variables with equal and unequal frequencies, the significance of differences was determined using the paired t-test and the independent t-test, respectively. Standardized Pearson coefficients were used to evaluate bivariate correlations. P values less than 0.05 and less than 0.01 were deemed statistically significant and highly statistically significant, respectively. To establish the cutoff value for

Zonulin, the receiver operating characteristic (ROC) analysis approach used. The area under curve (AUC) value was calculated using the ROC curve.

RESULTS

1-Demographic Characteristics of Patients and Control Group:

As shown in Table (1), 120 confirmed SARS-CoV-2 cases (59 males and 61 females) were included (50) cases (23 males and 27 females) in the mild/moderate category, (41) cases (21 males and 20 females) in the severe group and 29 cases (15 males and 14 females) in the critical category.

Table 1: Comparison of general characteristics between COVID-19 patient categories and the healthy group.

Parameters	COVID-19 patients group Mean ± SD			Healthy (60) Mean ± SD	P-value
	Critical (29)	Sever (41)	Mild/moderate (50)		
Sex, F/M	11/18	17/24	32/18	30/30	0.37
Age (years)	67.31±3.59	60.79±2.89	46.12±6.41	58.26 ± 5.76	A 0.10 B 0.20 C 0.10 D 0.06
BMI (kg/m²)	29.11±5.31	29.13.04	25.51±1.99	24.81 ± 3.43	A 0.61 B 0.85 C 0.31 D 0.85
SBP (mmHg)	169.0±5.91	149.17±4.83	153.01±2.16	132.83±6.52	A0.06 B 0.00 C 0.08 D 0.00
DBP (mmHg)	82.93±0.6	80.47±0.4	77.93±0.8	80.76 ± 4.89	A 0.00 B 0.01 C 0.07 D 0.60

Data represented as Mean ± SD: standard deviation, BMI: body mass index, SBP: Systolic blood pressure, DBP diastolic blood pressure F: females, M: male A= P.value (critical+ sever), B= P.value (critical + moderate) C P.value (sever+ moderate) D = (covid + healthy).

The general characteristics of the study groups are presented in Table (1) which consists data of the 120 Covid-19 patients, this group was divided to three cases (Mid/moderate, severe and critical) compared with (60) apparently healthy subjects. The baseline characteristics are non-significant in age between healthy and total covid.19 group. Systolic Blood Pressure was significant between the healthy and covid.19 group. In the current study, critical covid-19 patient group has higher age than severe and mild groups.

When compared to the control group's age (58.26 5.76 years), the patients' mean ages (67.31 3.59 years, 60.79 2.89 years, and 46.12 6.41 years) according to COVID-19 severity were not statistically different. Patients with severe infections of COVID-19 have mean BMIs that are significantly higher (29.13.04) than those with critical and mild-to-moderate disease (29.11-5.31 and 25.51-1.99 kg/m2, respectively) in comparison. In reality, more than half of mild/moderate cases were reported as being under 50, whilst severe and critical cases were documented as being above 50. Males are more likely to experience critical and severe sickness than females, with the exception of the moderate category (females more than males).

Table 2: Comparison of serum level of biochemical and laboratory test results of patients with COVID-19 parameters in covid-19 patients groups with control group.

Parameters	Critical Group (29) Mean ± SD	Sever Group (41) Mean ± SD	Moderate group (50) Mean ± SD	healthy group (60) Mean ± SD	P-value	T-test Value
TG (mg\dL)	283.41±2.66	282.34±1.23	237.03±0.62	135.83±1.52	a=0.00 b=0.00- c=0.00	a-51.63 b-68.97 c-52.21
TC (mg\dL)	156.45±2.26	167.19±1.99	178.01±1.69	179.64±1.87	a=0.00 b=0.000 c=0.891	a-7.18 b-3.706 c--0.138
LDL-C (mg\dL)	70.63±2.32	94.97±1.79	77.51±2.46	102.68±1.92	a=0.00 b=0.00 c=0.00	a-10.58 b-2.77 c-8.11
VLDL-C (mg\dL)	56.68±0.532	56.46±0.247	47.4±0.124	27.16±0.304	a=0.00 b=0.00 c=0.00	a--51.639 b--68.97 c--52.216
HDL-C (mg\dL)	29.13±0.816	33.208±1.63	35.62±0.323	47.79±0.93	a=0.00 b=0.00 c=0.00	a-14.02 b-8.293 c-10.285
CRI-1 (TC/HDL)	5.093±1.23	4.47±0.732	5.141±0.98	2.30 ± 0.68	a-0.06 b-0.215 c-0.00	a-1.88 b--1.262 c-4.137
CRI-11 (LDL/HDL)	2.291±0.83	2.478±0.424	2.566±0.506	1.119 ± 0.50	a-0.388 b-0.55 c-0.334	a--0.874 b--0.589 c-0.978
AIP (log TG/HDL)	0.079±0.02	0.05±0.011	0.073±0.018	0.401 ± 0.19	a-0.00 b-0.00 c-0.00	a-4.137 b--0.664 c--3.193
Iron conc (µg/dL)	40.95±3.63	41±3.57	48.15±3.95	100.19±5.99	a-0.00 b-0.00 c-0.00	a-7.37 b-7.23 c-6.39
zinc conc (µg/mL)	127.3±7.55	111.05±10.55	122.75±10.03	84.22±3.32	a-0.00 b-0.00 c-0.00	a- -5.87 b- -2.9 c-4.26
Ca (mg/dl)	9.11±0.135	8.51±0.23	8.7±0.134	9.56±0.133	a-0.02 b-0.00 c-0.00	a- 2.24 b-4.17 c-4.33
Na (mmol/L)	134.2±1.18	140.52±2.136	136.35±1.94	139.48±0.66	a-0.00 b-0.579 c- 0.08	a-4.19 b- -0.558 c-1.78
K (mmol/L)	3.95±0.122	6.42±1.87	4.21±0.211	3.9±0.23	a- 0.78 b- 0.097 c- 0.352	a- -0.163 b- -1.695 c- -0.939
Hb %	12.66±0.29	12.5±0.27	13.07±0.273	12.78±0.22	a- 0.74 b- 0.423 c- 0.408	a-0.329 b-0.808 c--0.834
T-WBC %	13.6±0.25	11.61±0.29	10.65±0.37	9.05±0.194	a- 0.00 b- 0.00 c- 0.00	a- -14.51 b- -7.58 c- -4.13
Neut %	9.8±0.47	8.67±0.4	6.15±0.43	5.86±0.3	a- 0.00 b- 0.00 c- 0.00	a- -7.35 b- -5.63 c- -0.575
Lymph. %	2.49±0.13	2.8±0.181	4.22±0.192	4.2±0.08	a- 0.00 b- 0.00 c- 0.911	a- 11.77 b-7.85 c- -0.113
Plt. (10^9/L)	309.07±8.59	251.6±11.25	221.82±13.9	294.4±5.35	a- 0.133 b- 0.00 c- 0.00	a- -1.52 b- 3.799 c- 5.539
CRP(mg/L)	42.67±1.84	30.53±2.13	29.06±1.763	3.36±0.25	a- 0.00 b- 0.00 c- 0.00	a- -25.63 b- -15.42 c- -17.53
D-Dimer (ng/ml)	4188.21±198.73	2796.15±14862	1438.33±80.107	289.43±251	a- 0.00 b- 0.00 c- 0.00	a- -23.72 b- -20.116 c- -15.951
Ferritin (ng/ml)	738±20.09	503.75±19.46	453.8±13.87	130.66±9.2	a- 0.00 b- 0.00 c- 0.00	a- -30.34 b- -19.04 c- -19.99
Zonulin (ng/mL)	326.4±12.46	313.46±11.16	424.18±89.25	79.69±11.77	a- 0.00 b- 0.00 c- 0.00	a--13.853 b- -13.685 c- -4.68

Data represented as mean ± SD, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein-cholesterol VLDL; C: Very Low-Density Lipoprotein- Cholesterol, TC: Total cholesterol; CRI.1: (TC/HDL) Castelli Risk Index; CRI-11: (LDL/HDL) Coronary risk index; AIP: (log TG/HDL-C) Atherogenic index of plasma. Data represented as Mean ± SD: standard deviation, Hb: hemoglobin, WBC: Wight blood cell, LYM: lymphocyte, NEUT: neutrophil, N/L: neutrophil/ lymphocyte, PLT: Platelet; a= p-value (critical + control); b= p-value (severe +control); c= p-value (mild + control).

The data of lipids profile consist of TG, VLDL-C levels were significantly higher (283.41±2.66), (56.68±0.532) respectively and HDL-C, LDL-C levels were significantly lower (29.13±0.816), (70.63±2.32) respectively in compared with healthy group (Table 2). The data of Iron conc, zinc conc were significant high and Ca conc were significant low when comparison with healthy group. The data of serum ferritin, D-

Dimer levels were significantly higher (738±20.09) and (4188.21±198.73) respectively.

The level of serum Zonulin compared with healthy group was significantly higher. In the severe patients, serum Zonulin was higher than that of critical and moderate patients groups (110.25±3.99), (96.8±3.27) and (82.82±11.57) respectively according to laboratory data from 120 patients. Additionally, major coagulation indicators, including D-dimer, were significantly raised in covid-19 patients, especially in circumstances when the condition was severe.

Table (3) show that no significant differences in serums Zonulin levels when compare between COVID-19 categories patient groups.

Table 3: comparison between general data and laboratory test results between COVID-19 categories patient groups.

Parameters	Critical Group (29) Mean ± SD	Sever Group (41) Mean ± SD	Moderate group (50) Mean ± SD	P-Value	T-test Value
TG (mg/dL)	283.41±2.66	282.34±1.23	237.03±0.62	a- 0.717 b- 0.00 c- 0.00	a- 0.365 b- 16.97 c- 32.67
TC (mg/dL)	156.45±2.26	167.19±1.99	178.01±1.69	a- 0.001 b- 0.00 c- 0.00	a- -3.55 b- -7.62 c- -4.13
LDL-C (mg/dL)	70.63±2.32	94.97±1.79	77.51±2.46	a- 0.04 b- 0.00 c- 0.00	a- -2.03 b- -8.29 c- -5.72
VLDL-C (mg/dL)	56.68±0.532	56.46±0.247	47.4±0.124	a- 0.717 b- 0.00 c- 0.00	a- 0.365 b- 16.97 c- 32.67
HDL-C (mg/dL)	29.13±0.816	33.208±1.63	35.62±0.323	a- 0.03 b- 0.00 c- 0.155	a- -2.22 b- -7.39 c- -1.45
Iron conc (µg/dL)	40.95±3.63	41±3.57	48.15±3.95	a- 0.78 b- 0.45 c-0.53	a- -0.282 b- -0.765 c- -0.635
zinc conc (µg/mL)	127.3±7.55	111.05±10.55	122.75±10.03	a- 0.30 b- 0.76 c- 0.42	a- 1.075 b- 0.306 c- -0.825
Ca (mg/dl)	9.11±0.135	8.51±0.23	8.7±0.134	a- 0.049 b- 0.048 c-0.535	a- 2.104 b- 2.115 c- -0.632
Na (mmol/L)	134.2±1.18	140.52±2.136	136.35±1.94	a- 0.01 b- 0.37 c- 0.19	a- -3.077 b- 2.115 c- -0.632
K (mmol/L)	3.95±0.122	6.42±1.87	4.21±0.211	a- 0.19 b- 0.51 c- 0.23	a- -1.350 b- -0.665 c- 1.244
Hb % (g/dL)	12.66±0.29	12.5±0.27	13.07±0.273	a- 0.68 b- 0.30 c- 0.14	a- 0.412 b- -1.026 c- -1.48
T-WBC %	13.6±0.25	11.61±0.29	10.65±0.37	a- 0.00 b-0.052 c- 0.03	a- 5.15 b- 6.53 c- 2.006
Neut. %	9.8±0.47	8.67±0.4	6.15±0.43	a- 0.07 b- 0.00 c- 0.00	a- 1.18 b- 5.67 c- 4.32
Lymph. %	2.49±0.13	2.8±0.181	4.22±0.192	a- 0.16 b- 0.00 c- 0.00	a- -1.42 b- -7.47 c- -5.36
Plt. (10^9/L)	309.07±8.59	251.6±11.25	221.82±13.9	a- 0.00 b- 0.00 c- 0.00	a- 4.05 b- 5.32 c- 1.66
CRP (mg/L)	42.67±1.84	30.53±2.13	29.06±1.763	a- 0.00 b- 0.00 c- 0.60	a- 4.29 b- 5.32 c- 0.528
D-Dimer (ng/ml)	4188.21±198.73	2796.15±14862	1438.33±80.107	a- 0.00 b- 0.00 c- 0.00	a- 5.6 b- 12.83 c- 8.04
Ferritin (ng/ml)	738±20.09	503.75±19.46	453.8±13.87	a- 0.00 b- 0.00 c- 0.04	a- 8.37 b- 11.63 c- 2.09
Zonulin (ng/mL)	96.8±3.27	110.25±3.99	82.82±11.57	a- 0.440 b- 0.290 c- 0.230	a- 0.789 b- -1.099 c- -1.251

Data represented as mean ± SD, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein-cholesterol VLDL; C: Very Low-Density Lipoprotein- Cholesterol, TC: Total cholesterol; AIP: (log TG/HDL-C) atherogenic index of plasma. Data represented as Mean ± SD: standard deviation, Hb: hemoglobin, WBC: Wight blood cell, LYM: lymphocyte, NEUT: neutrophil, N/L: neutrophil/ lymphocyte, PLT: Platelet; a= p-value (critical + control); b = p-value (severe +control); c= p-value (mild + control).

Table (4) demonstrated there is high significant in serums Zonulin level when compare between male and female patients and control (314.56±73.74, 333.85±41.81and 81.36±85.77, 78.02±35.47) respectively (p< o.o5).

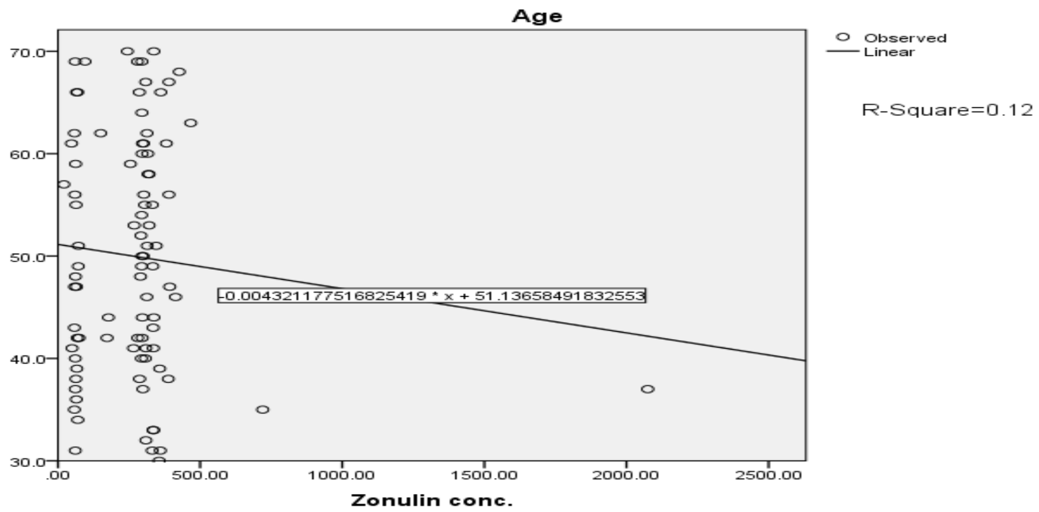
Table 4: Comparison of serum Zonulin level between males and females groups of patients and healthy.

Parameters	Group	Mean±S.D	T-test Value	P-Value
Zonulin conc. (ng/mL)	Male(control)	81.36±85.77	-8.603	0.00
	Male(patient)	314.56±73.74		
	Female(control)	78.02±35.47	-16.251	0.00
	Female(patient)	333.85±41.81		

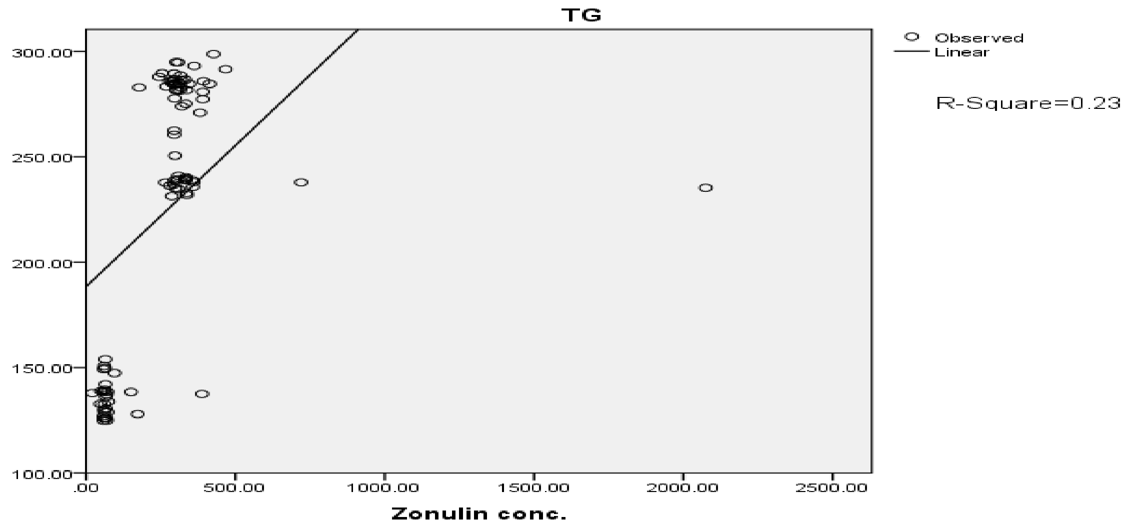
Table 5,6 and Figure 1, shown a significant correlation between serum Zonulin levels with Age, TG, TC, HDL-C, VLDL-C, LDL-C, Iron, Zinc, Ca, T-WBC, Lym, CRP, D-dimer , ferritin levels and Sensitivity and specificity of serum Zonulin levels in COVID-19 patients group.

Table 5: Correlation between serums Zonulin Level with clinical Parameters in COVID-19 patients group**.**

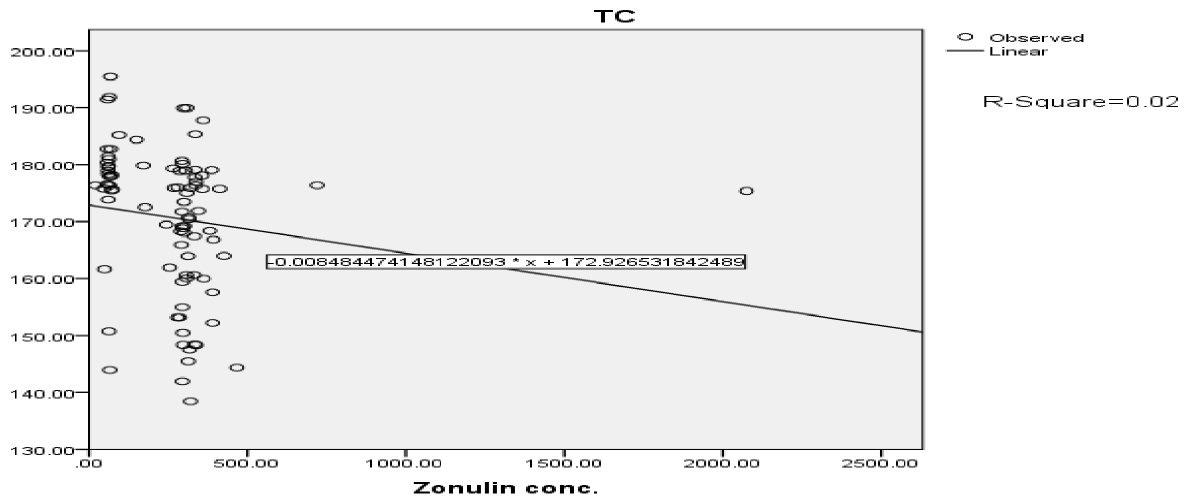
Parameters	r-	P. value
Age	0.293	0.050
BMI (kg/m2)	0.034	0.001
SBP (mmHg)	0.681	0.011
DBP (mmHg)	0.269	0.002
SpO2	0.877	0.000
TG (mg\dL)	0.278	0.008
TC (mg\dL)	-0.263	0.012
HDL-C (mg\dL)	-0.254	0.016
VLDL-C (mg\dL)	0.278	0.008
LDL-C (mg\dL)	-0.284	0.007
CRP-I (TC/HDL)	0.259	0.022
CRP-II (LDL/HDL)	0.201	0.029
Iron (µg/dL)	-0.431	0.000
Zinc (µg/mL)	0.304	0.004
Ca (mg/dl)	-0.211	0.046
Na (mmol/L)	0.041	0.702
K (mmol/L)	-0.040	0.708
Hb % (g/dL)	-0.099	0.353
T-WBC %	0.312	0.003
Neut. %	0.206	0.052
Lym. %	-0.250	0.018
Plt. (10^9/L)	0.122	0.250
CRP (mg/L)	0.228	0.031
D-Dimer (ng/ml)	0.031	0.000
Ferritin (ng/ml)	0.374	0.000


Age
Observed
Linear
R-Square=0.12
-0.004321177516825419 * x + 51.13658491832553
70.0
60.0
50.0
40.0
30.0
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

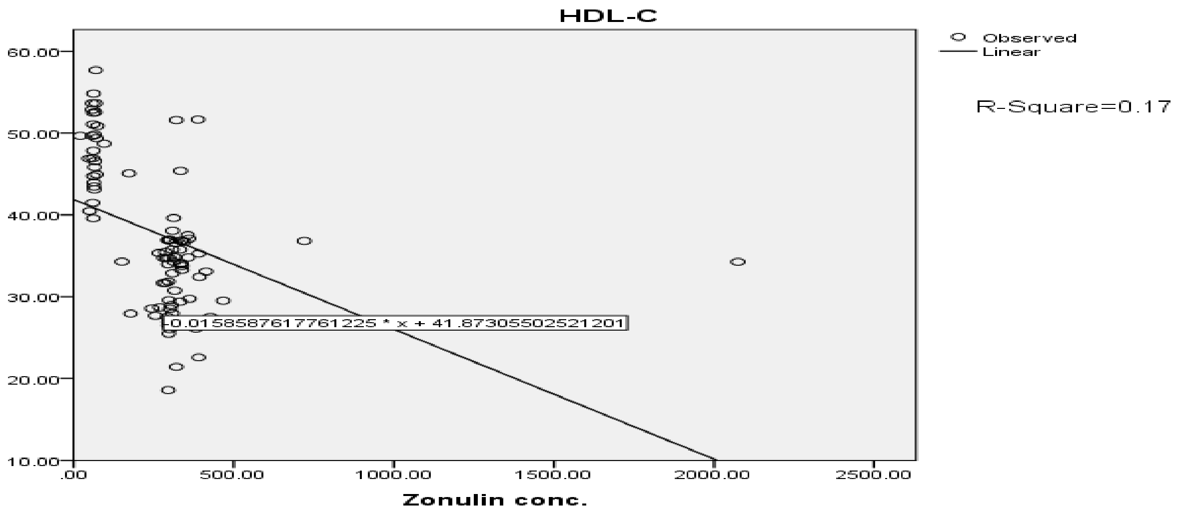
(A)


TG
Observed
Linear
R-Square=0.23
300.00
250.00
200.00
150.00
100.00
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

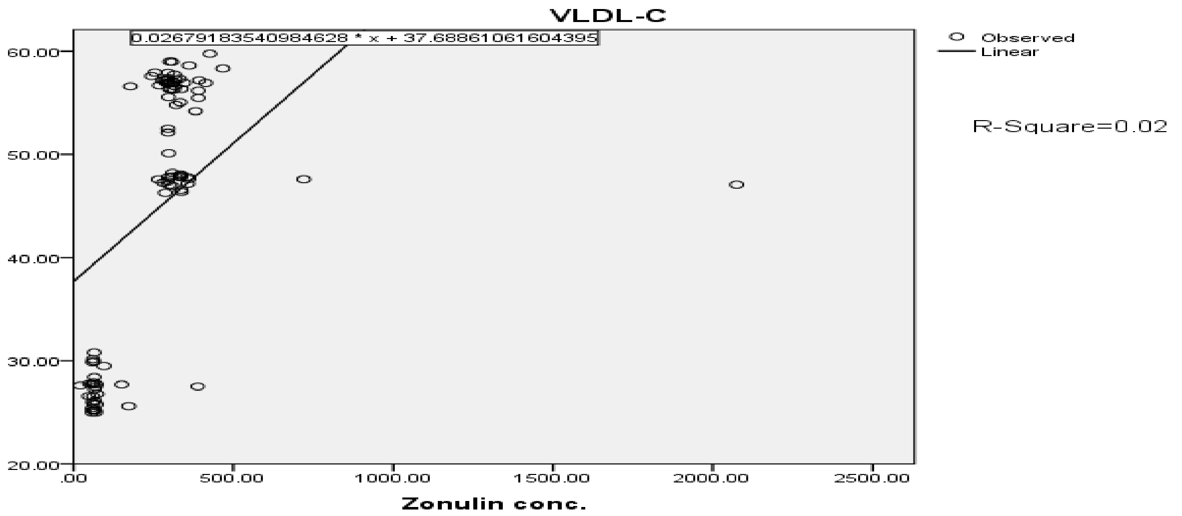
(B)


TC
Observed
Linear
R-Square=0.02
-0.008484474148122093 * x + 172.926531842489
200.00
190.00
180.00
170.00
160.00
150.00
140.00
130.00
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

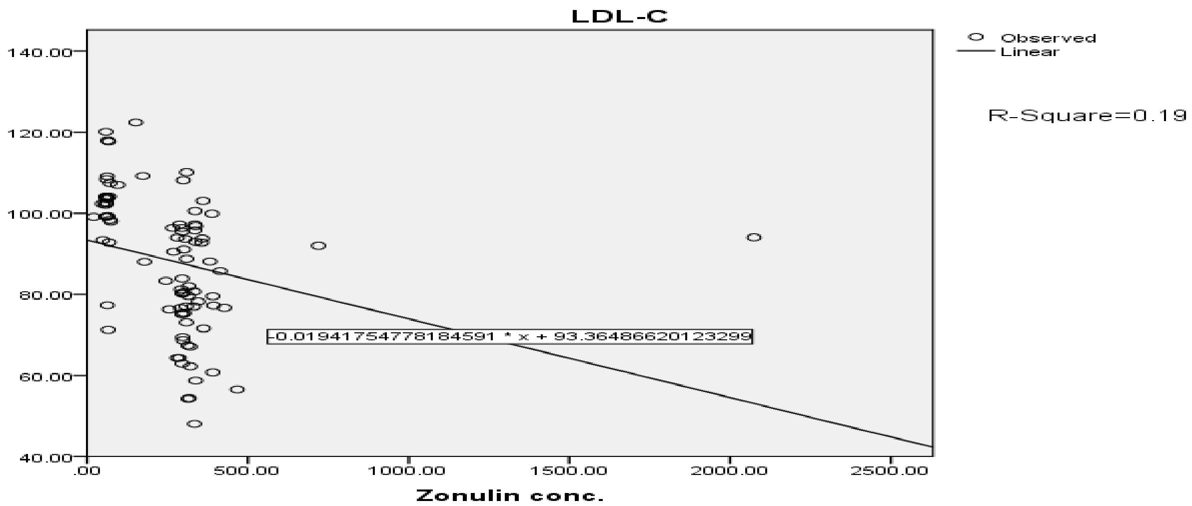
(C)


HDL-C
Observed
Linear
R-Square=0.17
-0.0158587617761225 * x + 41.87305502521201
60.00
50.00
40.00
30.00
20.00
10.00
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

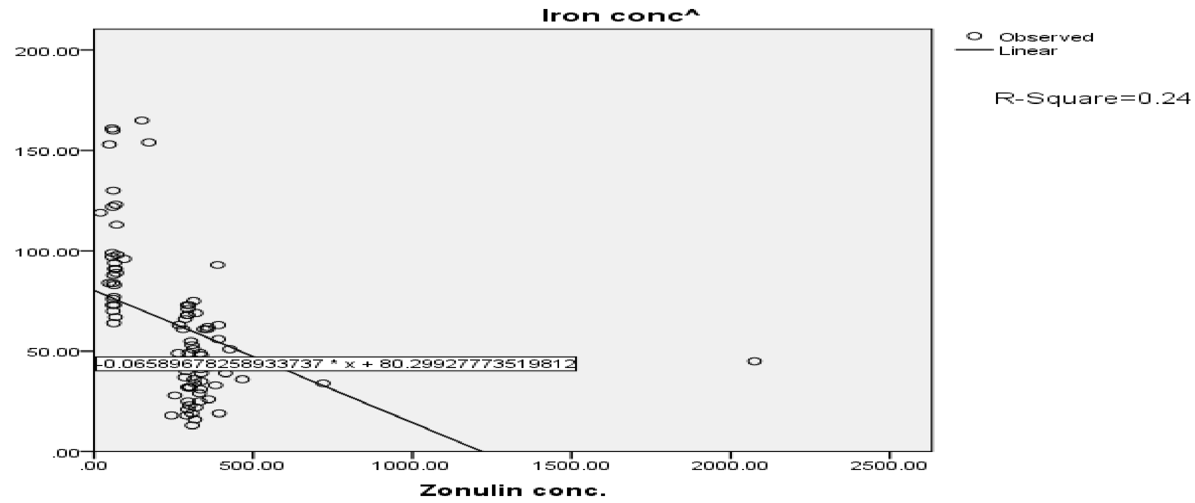
(D)


VLDL-C
Observed
Linear
R-Square=0.02
0.02679183540984628 * x + 37.68861061604395
60.00
50.00
40.00
30.00
20.00
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

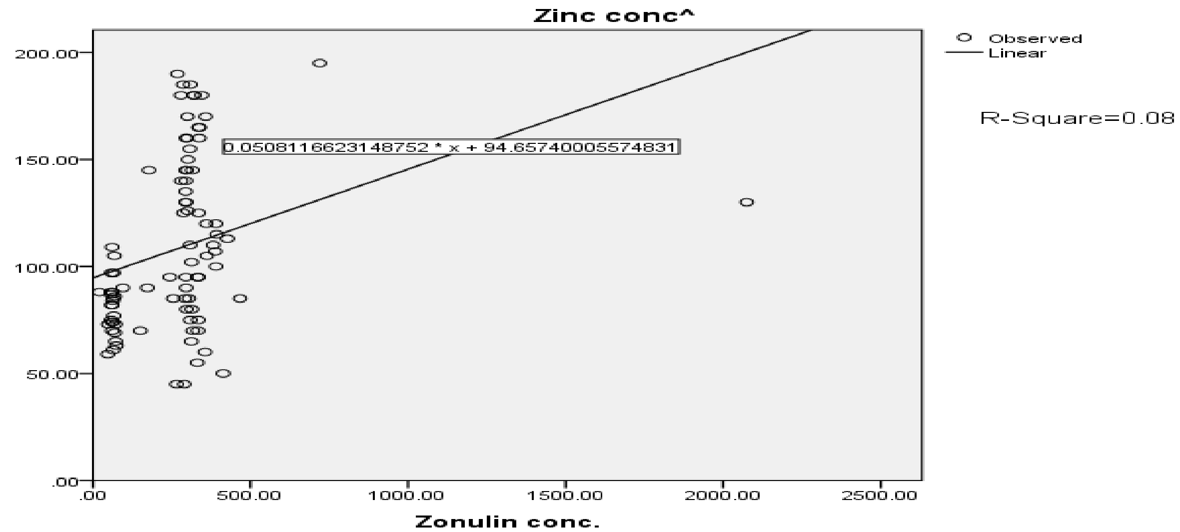
(E)


LDL-C
Observed
Linear
R-Square=0.19
-0.01941754778184591 * x + 93.36486620123299
140.00
120.00
100.00
80.00
60.00
40.00
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

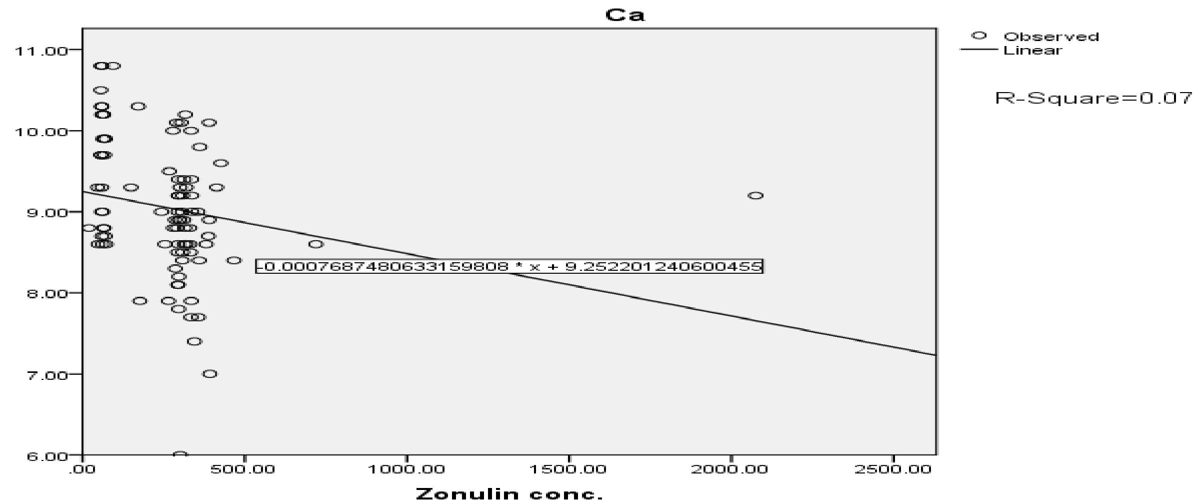
(F)


Iron conc^
Observed
Linear
R-Square=0.24
-0.06589678258933737 * x + 80.29927773519812
200.00
150.00
100.00
50.00
.00
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

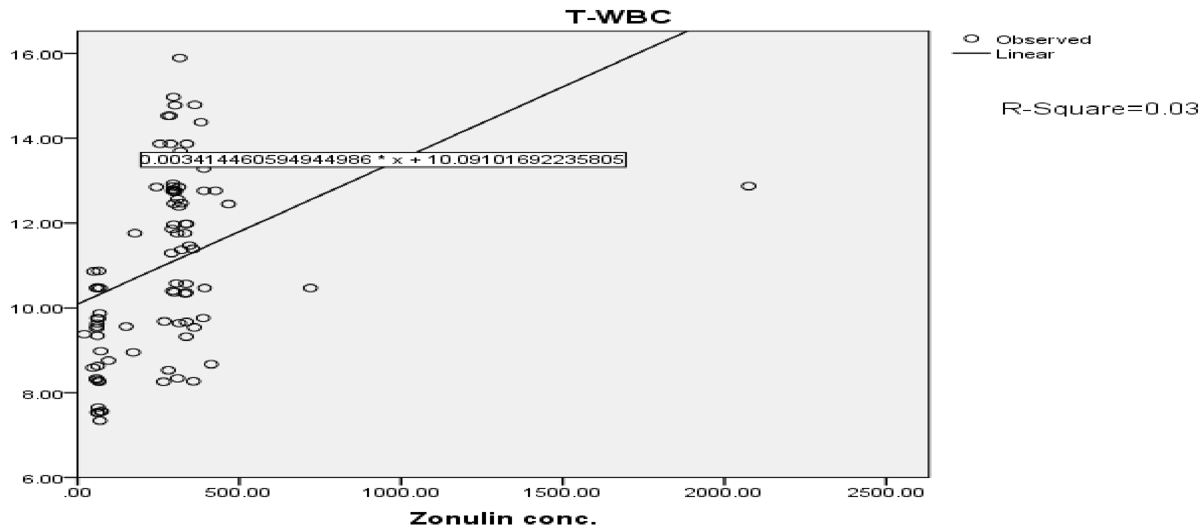
(G)


Zinc conc^
Observed
Linear
R-Square=0.08
0.0508116623148752 * x + 94.65740005574831
200.00
150.00
100.00
50.00
.00
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

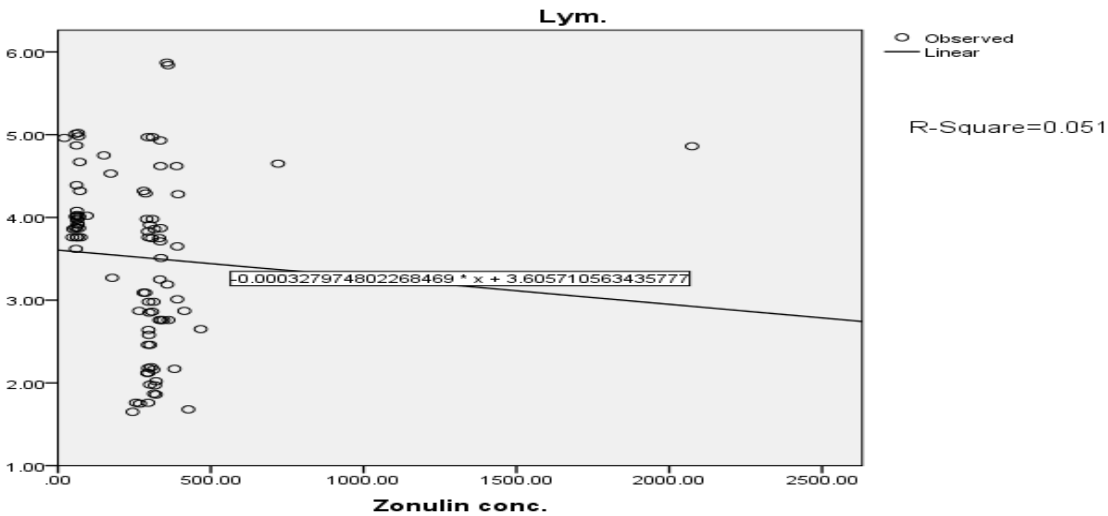
(H)


Ca
Observed
Linear
R-Square=0.07
-0.0007687480633159808 * x + 9.252201240600455
11.00
10.00
9.00
8.00
7.00
6.00
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

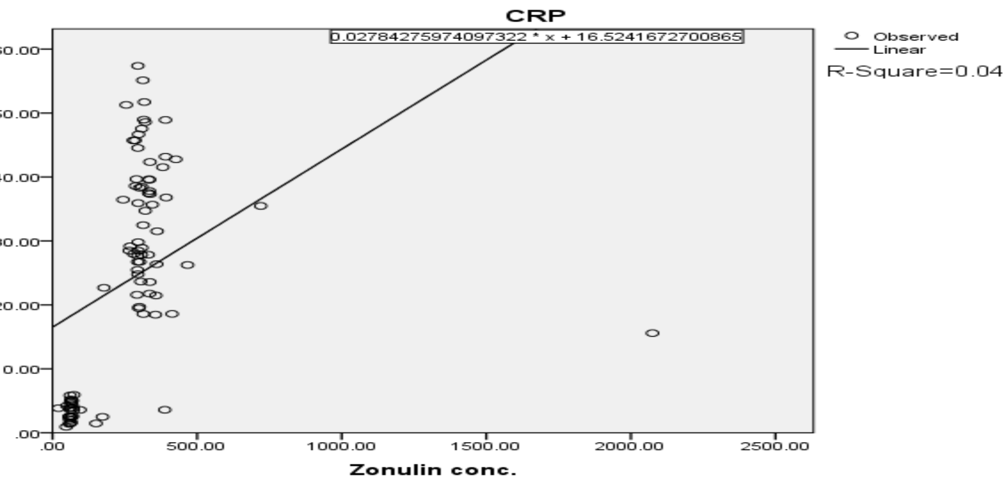
(I)


T-WBC
Observed
Linear
R-Square=0.03
0.0034144605949444986 * x + 10.09101692235805
16.00
14.00
12.00
10.00
8.00
6.00
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

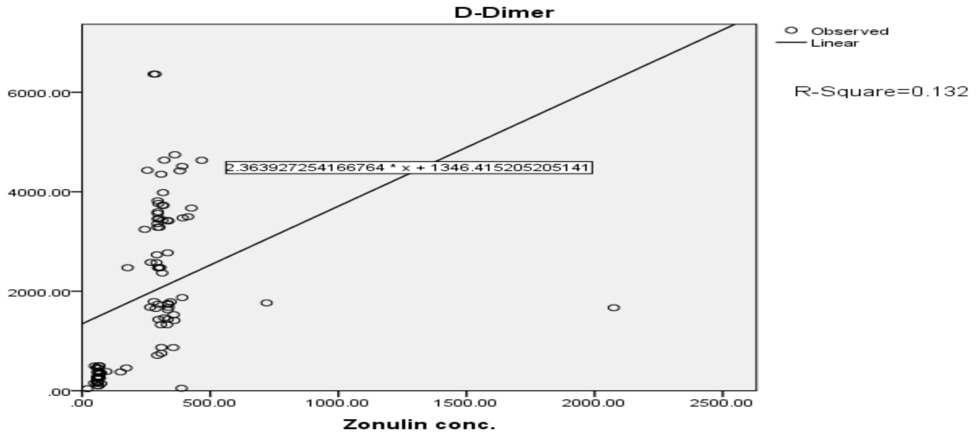
(J)


Lym.
Observed
Linear
R-Square=0.051
-0.000327974802268469 * x + 3.605710563435777
6.00
5.00
4.00
3.00
2.00
1.00
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

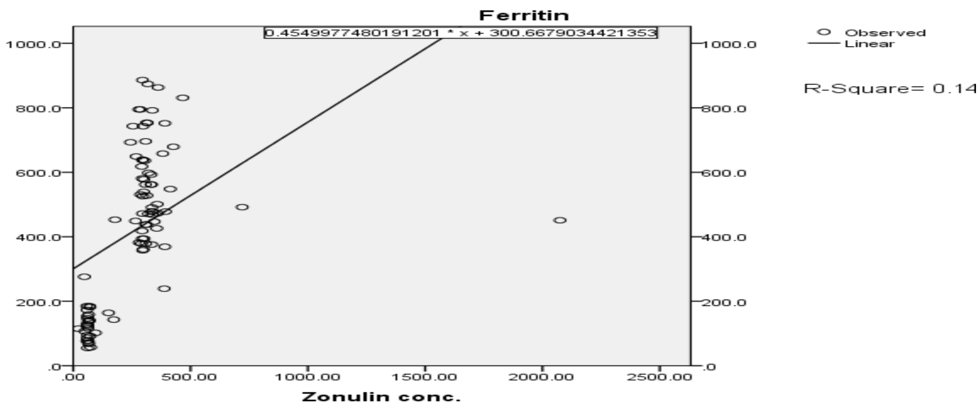
(K)


CRP
0.02784275974097322 * x + 16.5241672700865
Observed
Linear
R-Square=0.04
60.00
50.00
40.00
30.00
20.00
10.00
.00
.00
500.00
1000.00
1500.00
2000.00
2500.00
Zonulin conc.

(L)



(M)



(N)

Fig. 1: Correlation between Zonulin levels with (A) Age, (B) TG, (C) TC, (D) HDL-C, (E) VLDL-C, (F) LDL-C, (G) Iron, (H) Zinc, (I) Ca, (J) T-WBC, (K) Lym, (L) CRP, (M) D-dimer and (N) ferritin in COVID-19 patients.

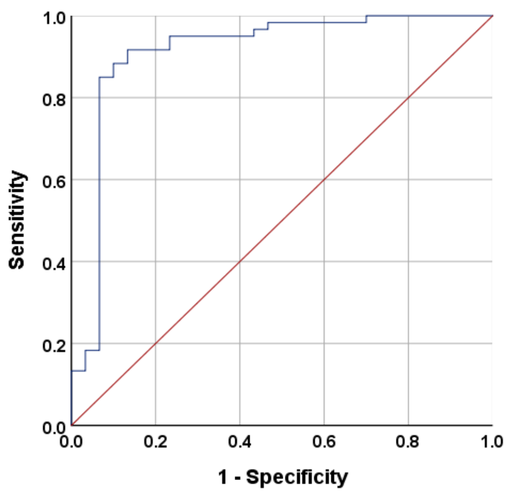


Fig. 1: ROC curve of Zonulin levels

Table 6: Sensitivity and specificity of serum Zonulin levels in COVID-19.

Variable	Cut-off concentration	Sensitivity %	Specificity %	AUC	95% CI of AUC	p-value
Zonulin (ng/mL)	276.812	91.7	89.2	0.912	0.833-0.991	<0.0001

DISCUSSION

A family of viruses known as the Coronaviridae includes the coronaviruses. Alpha-coronaviruses, beta-coronaviruses, gamma-coronaviruses, and delta-coronaviruses are the four genera that make up this viral family (Han Y 2019). The alpha-coronavirus and beta-coronavirus genera are where human coronaviruses (HCoVs) originate. Both upper and lower respiratory tract infections are brought on by SARS-CoV-2. The infection progresses from asymptomatic or mild sickness to severe systemic symptoms primarily affecting the lung and digestive system, and lastly to critical symptoms that result in multi-organ failure (Wiersinga WJ 2019).

Growing research indicates that COVID-19 severity and mortality are correlated with poor immune response and hyper-inflammatory response (Li X 2020). Severe COVID-19 is frequently associated with metabolic problems such as sepsis(the body's extreme response to an infection, it is a life-threatening medical emergency) and systemic inflammation (Chidambaram V 2022).Patients with SARS-CoV-2 also experience gastrointestinal symptoms (such as fever, myalgia, lethargy, dry cough, dyspnea, anorexia, abdominal pain, and diarrhea) While respiratory symptoms are the second most common in COVID-19 patients, gastrointestinal symptoms are among the most prevalent. (Zhu N 2019) Viral interactions with cells are intricate. Recent research indicates that cellular lipids are crucial for viral replication, fusion of the viral membrane with the host cell membrane, and endocytosis and exocytosis (Theken KN 2021). According to Van Lenten *et al.* (1995) and Khovidhunkit *et al.* (2004), HDL can cause either an inflammatory or an anti-inflammatory profile. (Femlak M 2017) Patients with infections and sepsis had lower HDL plasma levels (Cirstea et al., 2017). (Feingold KR 2016) A change in lipid profile can serve as a good early warning sign for COVID-19 disease severity (moderate or severe) (Nie *et al.*, 2020). (Cirstea M 2017)

This indicates that our investigation has found a direct relationship between fatal clinical outcomes in COVID patients and lipid profiles. Changes in lipid profiles have been researched as potential biomarkers to aid in the trigger-based identification of viral infections. According to studies, patients with COVID-19 had reduced levels of TC and HDL-C but not TG or LDL-C, which is consistent with our findings. This conclusion is supported by the findings of past experiments (Li G. *et al.*, 2020). The serum/plasma concentrations of total TC and HDL-C, but not TG, were considerably lower in COVID-19 patients with more serious diseases.

The major conclusions are consistent with research by Sun J et al, which showed that individuals with COVID-19 who had severe illness had lower HDL-C levels. (Sun, J 2020) Huang C et al. show how low levels of apoA-1 and HDL-C at admission were able to predict the severity of COVID-19 and mortality during hospitalization. (Huang C 2020) Throughout the course of the disease, levels of apoA-1 and HDL-C significantly strayed from the normal reference range and were closely linked with inflammatory markers. (Femlak M 2017)

One of the aberrant laboratory values reported in patients with COVID-19 infection is elevated D-dimer and ferritin. An elevated D-dimer level indicates hyperfibrinolysis and excessive coagulation activation. As a result, D-dimer has a high sensitivity but a low specificity for the detection of active thrombus (Feingold KR 2016).

According to our findings, COVID-19 patients with elevated levels of D-dimer and ferritin had a higher probability of contracting a serious infection and dying from any cause than those with normal levels. The levels of D-dimer were higher in patients with severe COVID-19 infection and those who acceded to death, compared with non-severe disease and those who survived. The present study find elevated D-dimer, ferritin, CRB and neutrophil in severe COVID-19 patients when compared with non-severe. It is frequently used to diagnose disseminated intravascular coagulation in people with low and moderate pretest probabilities for deep vein thrombosis (DVT) and pulmonary embolism (PE). D-dimer is the fibrin breakdown product generated upon cleavage of cross-linked fibrin by plasmin (Verity R 2019).

As is consistent with our findings, Weitz JI et.al showed that increasing d-dimer levels during hospitalization were linked to lower long-term outcomes. (Weitz JI 2017) D-dimer has recently been researched to find people who are expected to acquire severe COVID-19 infection earlier in the course of their illness. (Zhou F 2020) Inflammatory/ infection markers (ESR, CRP, LDH, and PCT, but not IL-6), coagulation function tests (fibrinogen, PT, and D-dimer), and glucose were positively associated with the COVID-19 severity. According to other investigations, SARS-CoV-2 infection causes lymphocytopenia. (Lippi G 2020). Additionally, recent research found that in severe cases, neutrophil counts were slightly elevated but lymphocyte, eosinophil, and monocyte counts were all lowered. (Koch V 2021) In severe cases of COVID-19, lymphopenia as a dysregulation in the immune response is mapped by a greater loss in T cells, particularly T helper cells (Qin C 2020).The role of Zonulin as a predictor of COVID-19 infection severity was investigation in the current study.

Zonulin has mainly localized in the Gastrointestinal (GI) tract and connected to GI diseases like coeliac disease (de Lemos 2003). Our results found high significant increase in serum levels of Zonulin in critical group than in severe and moderate group, and also found significant increase in serum levels of Zonulin in COVID-19 patients when compared with healthy group. Numerous indicators suggest that zonulin may be involved in the neurological effects of SARS-CoV-2 infection: zonulin may function in the brain despite BBB damage. A new study employing the zonulin agonist peptide AT-1002 demonstrates that zonulin is linked to enhanced BBB permeability, providing evidence in favor of this theory (Losy J 2021). The BBB's permeability being made more permeable is a typical virus damaging mechanism (Fantini J 2020). In comparison to COVID-19 patients without neurological problems, COVID-19 patients with neurological disorders have a higher risk of in-hospital death and lower rates of discharge home (Robinson CP 2020).

In a different study, Simone Di et al. found that zonulin is involved in the pathogenesis of acute lung injury (ALI) in mouse models and that its peptide inhibitor, larazotide acetate (also known as AT1001), reduced the severity of ALI and subsequent mortality by reducing mucosal permeability to fluid and neutrophil extravasation into the lungs. (Di Micco S 2021).

The emergence of interstitial pneumonia causing (ALI) and/or (ARDS), both of which are fatal when infected people, is the most severe consequence of COVID-19 infection (Ghahramani S 2020). There are few treatments available for acute lung injury (ALI) and acute respiratory disease syndrome (ARDS) in this patient population, and there is no established and effective treatment for SARS-CoV-2 infection at this time. According to a recent review research of 109 COVID-19 patients in China who had ARDS (Zhou et al., 2020), those with moderate and severe ARDS had greater mortality rates, and there was no discernible benefit of antiviral, glucocorticoid, or immunoglobulin treatment on survival. (Zhou F 2020)

The limitations of our study should be taken into account when interpreting the findings. The sample size was firstly somewhat small. Second, because this was a study with a single measurement and no patient follow-up over time or observation of how Zonulin behavior evolved, we are planning a cohort study in which Zonulin will serve as the baseline measurement and include following-up on the variables of interest, giving the findings of this study more significance.

Conclusions

The management of problems, the prognosis, and the discharge of patients from hospital settings are all significantly influenced by biomarkers. Biomarkers must be substantially included clinical processes and treatment decision-making in addition to clinical assessment. Serum Zonulin > 326.4 ng/ml, therefore it the factor that should be actively watched for COVID-19 progression of the critical stage and perhaps averted. Other predictive factors include ferritin, serum potassium, high-density lipoprotein cholesterol, lymphocyte count, and serum potassium.

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